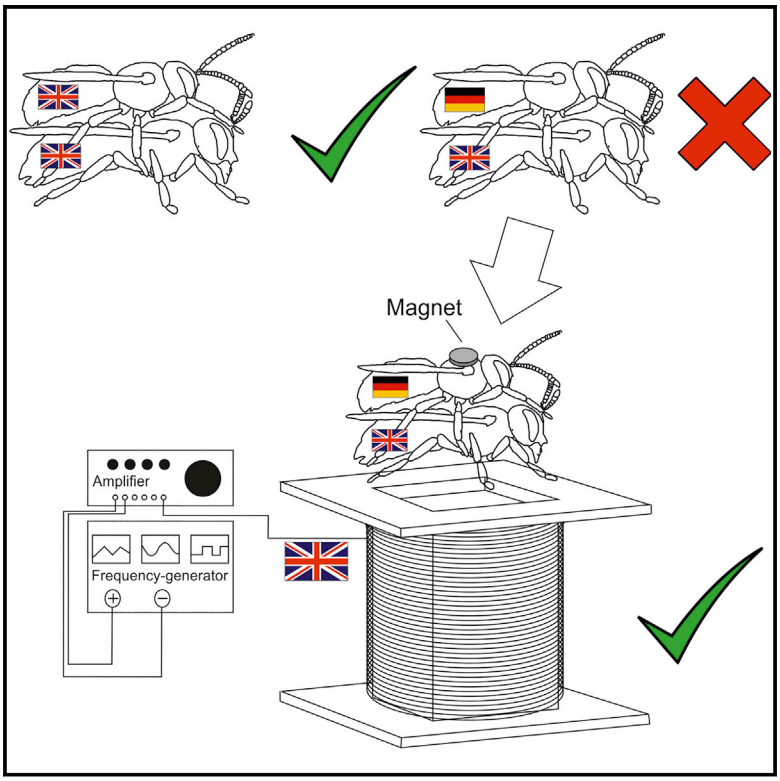
Report

Current Biology

The Role of Vibrations in Population Divergence in the Red Mason Bee, *Osmia bicornis*

Graphical Abstract



Highlights

- Females of *O. bicornis* prefer to mate with males from their own region
- Vibrations are the main signal for females to distinguish between different regions
- Our newly developed bioassay allows us to impose a novel signal onto a live animal
- We have discerned the first possible steps toward speciation in this species

Authors

Taina Conrad, Manfred Ayasse

Correspondence

taina.conrad@gmx.de (T.C.),
manfred.ayasse@uni-ulm.de (M.A.)

In Brief

Conrad and Ayasse investigate whether female red mason bees show selective mate choice between males from different regions based on a male's vibrations. With an exciting new bioassay, they show that females prefer mating with males from their own region using male vibrations as a signal, indicating that there is divergence between populations.

Conrad & Ayasse, 2015, Current Biology *25*, 2819–2822
November 2, 2015
http://dx.doi.org/10.1016/j.cub.2015.08.059

CellPress

Current Biology
Report

The Role of Vibrations in Population Divergence in the Red Mason Bee, *Osmia bicornis*

Taina Conrad[1,*] and Manfred Ayasse[1,*]
[1]Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Helmholtzstraße 10-1 Containerstadt, 89081 Ulm, Germany
*Correspondence: taina.conrad@gmx.de (T.C.), manfred.ayasse@uni-ulm.de (M.A.)
http://dx.doi.org/10.1016/j.cub.2015.08.059

SUMMARY

Differences in female preference for certain male characteristics can be a driving force for population divergence and speciation [1–4]. During precopulation, females of the red mason bee, *Osmia bicornis*, choose suitable males based on, among other criteria, their thoracic vibrations [5]. These vibrations are thought to be a signal of a male's fitness with females choosing the strongest males that can vibrate for the longest time [5]. The precise role of such vibrational signals, however, has not been determined by bioassays, and the vibrations might also play a role in species recognition [6]. There are two main subspecies of *O. bicornis* in Europe distinguishable only by a single morphological trait [7] (Figure S1). We therefore developed a new bioassay allowing us to impose the vibrations of one live male onto another in order to discern possible selective mate choice by females from *O. bicornis* originating from different regions of Europe. Females showed strong preference for males from their own region, and male vibrations were the main signal involved in this choice. Thus, vibrational signals encode not only fitness but also information about the region of origin indicating that divergence exists between the different European *O. bicornis* populations, which might ultimately lead to speciation. These results provide new insights into the scope of vibrational communication in bees, a group previously considered to rely predominantly on chemical signals [8, 9]. Our newly developed method should shed further light on many exciting questions concerning vibrational communication in bees and other animal taxa.

RESULTS AND DISCUSSION

Cross-mating experiments in the laboratory with bees of the two subspecies of *Osmia bicornis* from Germany, the UK (Figure S1), and with bees from Denmark showed that all tested females were highly attractive to males and that almost immediately a male established itself on each female's back, showing precopulatory behavior.

These cross-mating experiments clearly revealed that females prefer to mate with a male from their own region over a male from another region (Table 1; see also Figure S1) (Wald chi-square, $\chi^2 = 30.933$, degrees of freedom [df] = 8, $p < 0.01$). The finding that the fewest matings occurred between females from England and males from Germany could be explained by a divergence between the two subspecies observed by Peters [7] and an ongoing speciation event. Speciation is usually a long process [3], and we might thus be looking at the first steps of a separation between the different regions. As females prefer to mate with males from their own region, this might lead to a continuous separation of the different subspecies based on intersexual selection, as has been shown in other insects [10–12].

If male vibrations during mating are solely a sign of fitness, we would expect the males with the longest vibrations to be chosen regardless of other signal parameters such as frequency or modulation range, since these do not relay information about fitness [5]. Female preference for local males could then depend on other signals such as the males' odor. To test this, however, one would have to change the vibrations of a live male without changing any of its chemical or visual cues. We, therefore, developed a bioassay enabling us to achieve this by putting a small magnet on the thorax of the male. The mating pair in precopulation was then placed on an inductor that was connected to a frequency generator emitting a pre-recorded male signal. The signal was transferred through the electromagnetic field to the magnet, and the male then vibrated in the desired frequency (Figure 1; see also Figure S2). After the imposition of another signal, most males stopped emitting their own vibrations, or their innate signals were overpowered by the magnet's vibrations. All the males otherwise continued their usual mating behavior and attempted to copulate.

We tested this setup with the combination that was least successful in the cross-matings and transferred the signal of an English male onto a German male mating with an English female. The mating success of the German males increased significantly and did not differ from the success of local males (Wald chi-square, $\chi^2 = 13.804$, df = 2, $p < 0.05$; q values < 0.05) (Figure 2). The same occurred for English males fitted with a German signal and mating with a German female (Wald chi-square, $\chi^2 = 7.801$, df = 2, $p < 0.05$; q values < 0.05) (Figure 3).

Since this effect could have been attributable to an unspecific positive effect of the inductor alone, we also ran a control test with German males trying to mate with German females while we imposed an English signal. As expected, the success rate was significantly reduced (2 × 2 contingency table, df = 1, $p < 0.05$) (Figure S3).

These experiments show that females are indeed able to detect regional differences in the vibrational signals of the males and, furthermore, use them to discriminate between males.

Table 1. Mating Success of Males from Different Countries

	Germany (♂)	England (♂)	Denmark (♂)
Germany (♀)	a, 90%, n = 20	cd, 44%, n = 32	cd, 40%, n = 20
England (♀)	d, 20%, n = 20	ab, 83%, n = 18	bc, 60%, n = 20
Denmark (♀)	cd, 40%, n = 20	cd, 46%, n = 11	ab, 80%, n = 20

Comparison of successful matings in different combinations of females and males of *O. bicornis* from three regions of Europe (Germany, England, and Denmark). The sample sizes (n) are shown. Significant differences between groups are shown by different letters (generalized linear model pairwise, q values, $p < 0.05$).

Female choice could thus drive speciation, as has been found in cichlid fish in the great lakes of eastern Africa [13]. Further analysis of the vibrations produced by males from the different regions should demonstrate which parameters of the signal are central to this choice.

Our results clearly demonstrate selective mate choice and that females show a strong preference for males from their own region. This preference could be significantly altered by changing the male's vibrations. We therefore conclude that vibrational signals in *O. bicornis* males encode not only fitness, as previously thought, but also information about their region of origin, indicating that there is already divergence between different European *O. bicornis*. The communication signal differences might ultimately lead to speciation. In most of the insect species investigated to date, chemical signals such as sex pheromones are thought to play a major role in mate attraction [8], species isolation, and speciation [14, 15]. Our results shed new light on the important role that vibrational signals play in the mating of bees and subsequently in speciation.

Our new method also paves the way for further studies into vibrational communication. As this method is non-invasive, various studies are now possible in which the vibrations of a live individual can be changed without changing any of its other cues. This could lead to new insights into the vibrational communication employed by various species.

EXPERIMENTAL PROCEDURES

Study Animals

For this study, we used both subspecies of the solitary bee *O. bicornis* Linnaeus 1758 (Hymenoptera: Megachilidae), *O. bicornis rufa* and *O. bicornis cornigera* (see Supplemental Information for rearing information).

For the subspecies *O. bicornis rufa*, we received bees from natural populations in Regensburg, Constance, and Halle. For the subspecies *O. bicornis cornigera*, we used bees from Kent, Hereford, and Tonbridge. We also received cocoons from three natural populations in Denmark (Møn, Vejle, and Copenhagen), which represents a region in which both subspecies co-occur. To avoid misunderstandings, we refer to bees from one country (England, Germany, and Denmark) as bees from a region, because they are geographically close and not because of country borders.

During courtship, the male embraces the female while sitting on her back and engaging in a series of behavioral patterns in order to persuade the female to mate. The male vibrates his thorax, rubs himself against the female, while passing his antennae repeatedly over those of the female and his forelegs over the female's compound eyes [16, 17] (Movie S1). After this elaborate courtship, the female either accepts a male for copulation or rejects him and throws him off her back. Females choose a strong male that is able to vibrate for extended periods without interruptions [5].

Cross-Matings

We conducted cross-mating tests to establish whether bees from different regions are still able to mate with each other. For these tests, all nine combinations of males and females from the three regions were cross-mated with each

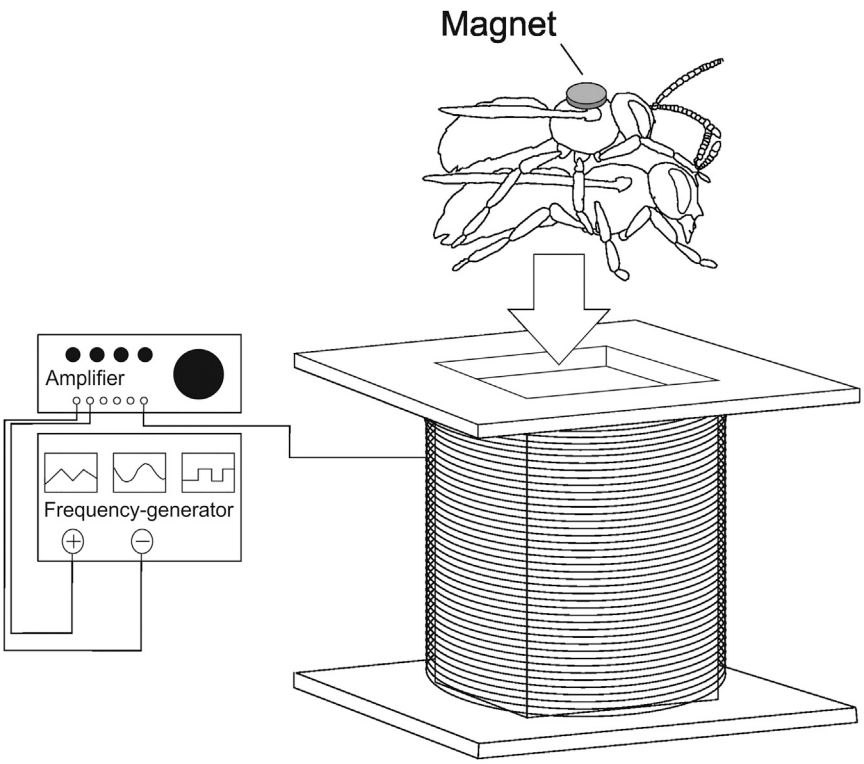


Figure 1. Setup of Our Bioassay

The pre-copulatory pair was placed on the iron core within the inductor. The signal of a successful male, produced by a frequency generator and then amplified, was then transmitted onto the magnet via the electromagnetic field. See also Figure S2.

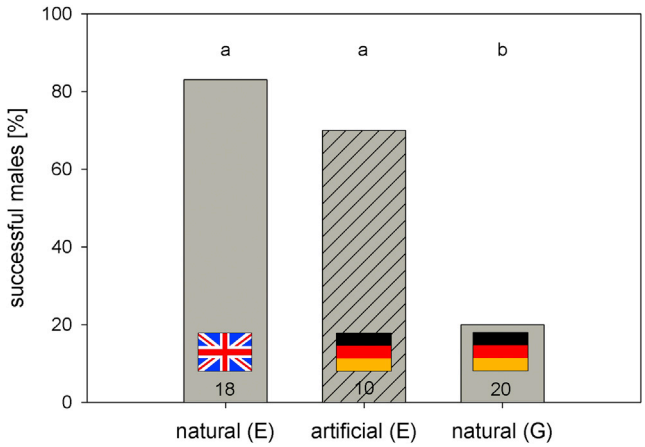


Figure 2. Mating Success of *O. bicornis* Males with English Females
Comparison of mating success of *O. bicornis* males of different origin (shown by flags) with a natural or an imposed artificial signal (E, English; G, German) trying to mate with English *O. bicornis* females. The sample sizes are shown beneath each flag. Significant differences are marked by different letters (Wald chi-square, $\chi^2 = 13.804$, df = 2, $p < 0.05$; q values < 0.05). See also Figure S3.

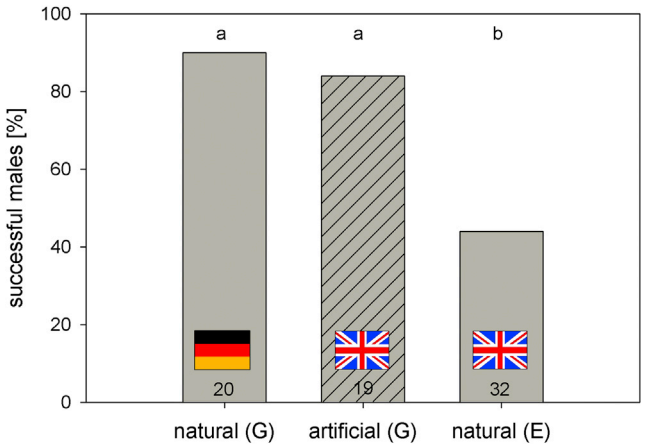


Figure 3. Mating Success of *O. bicornis* Males with German Females
Comparison of the mating success of *O. bicornis* males of different origin (shown by flags) with a natural or an imposed artificial signal (E, English; G, German) trying to mate with German *O. bicornis* females. The sample sizes are shown beneath each flag. Significant differences are marked by different letters (Wald chi-square, $\chi^2 = 7.801$, df = 2, $p < 0.05$; q values < 0.05). See also Figure S3.

other, and the number of successful males (males that were allowed to copulate) was measured (success rate).

Bioassays

In order to investigate whether female bees are able to recognize differences in vibrations produced by males, and whether they use these vibrations to discriminate between males of different regions, we conducted bioassays and developed a new method to impose one male's vibrations onto another live male.

In our setup, a small magnet (disc magnet ø 2 mm, height = 1 mm, 0.024 g, S02-01-N Material: NJFeBmagnetisation: N48 coating nickel [Ni-Cu-Ni], http://www.supermagnete.de) was glued onto the thorax of an English or German male by using resin. The male was then transferred into a flight cage with about ten females of the other respective region, and once a mating pair was established, it was placed on an inductor (300 loops, 0.8 Ω). A function generator (Agilent 33210A) connected to an amplifier (Pioneer A-105, Willich) was used to generate the signal of a successful German or English male. The electromagnetic field of the inductor then transferred the signal onto the magnet, and the male's thorax began to vibrate in synchrony with the signal (Figure 1).

The successful transference of the signal from the magnet to the male was tested with a laser vibrometer (Polytec PDV-100, Waldbronn) connected to a computer using a 32-bit sound card and Soundforge 9.0 software (SonicFoundry) at a sampling rate of 44.1 kHz. The files were later analyzed using Spike 2 (Cambridge Electronic Design). Most males stopped emitting their own vibrations, or their signals were overpowered by the magnet's vibrations (strength of the electromagnetic field: 0.4 mT). However, all males otherwise continued their mating behavior and, after a while, tried to copulate.

In a series of pre-trials, we found that the English signal consisted of a mean frequency sweep of 379.92 ± 86.58 Hz SD (n = 41) over a period of 300 ms and the German signal consisted of a mean frequency sweep of 402.01 ± 81.77 Hz SD (n = 57) over a period of 100 ms. Based on these results, in our bioassays, the parameters for both signals were taken from a successful male with values close to the mean frequency for that region. With this method, we were able to conduct 19 trials of English males with a German signal and 10 trials of German males with an English signal. We then compared the results to the respective mating success of males without an artificial signal. As a control, we also used German males with an artificial English signal on German females and English males with an artificial German signal on English females. We also tested whether either the magnet or the inductor alone had any effect but found no effect for either (n = 10; 2 × 2 contingency table, df = 1, $p < 0.05$).

Statistics

For statistical analysis of the data, we used the software SigmaStat 3.1 (Systat Software), Sigma Plot 9 (Systat Software), R (Development Core Team, 2009), and SPSS 13.0 (SPSS). All data were checked for deviation from a normal distribution by using a Kolmogorov-Smirnov test.

To compare the rates of successful matings between regions and between pairings with and without artificial signals, we used a binary logistic generalized linear model. To compare the mating success of the males with the various females, we examined our data with two models, i.e., using both the female and male origin separately (corrected Akaike's information criterion [AICC] = 47.501) or using the mating pair (AICC = 47.501) as a predictor. The region of origin of either the male or female did not predict mating success, but a highly significant interaction effect was observed (Wald chi-square, $\chi^2 = 28.620$, df = 4, $p < 0.01$). A highly significant effect was also seen when looking at the model for mating pairs (Wald chi-square, $\chi^2 = 30.933$, df = 8, $p < 0.01$). We then compared the different mating pairs using a pairwise comparison and a correction using q values. Different individuals were used for each series of tests of cross-matings and bioassays.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.08.059.

AUTHOR CONTRIBUTIONS

Conceptualization, T.C. and M.A.; Methodology, T.C. and M.A.; Investigation, T.C.; Resources, M.A.; Writing – Original Draft, T.C.; Writing – Review & Editing, T.C. and M.A.; Supervision, M.A.; Funding Acquisition, T.C. and M.A.

ACKNOWLEDGMENTS

We thank Dr. Axel Schmid for the initial idea of using magnets in the bioassay; Dr. Sebastian Breckerbohm for help with the physics; Dr. Tomás Murray for help with the statistics; Melanie Marquardt for her help collecting data; everyone who has provided us with bees from different countries, namely Robin Dean, Dr. Karsten Seidelmann, Dr. Eberhard Strohm, Sabine Rademacher, Mike Hermann, Henrik F Brødsgard, Anja Amtoft Wynns, and Adam Bates; Prof. Robert Paxton, Prof. Hannelore Hoch, and Dr. Roland Mühlethaler for comments on the manuscript; Dr. Cynthia Tedore, Prof. Ian Lawrance, and

Theresa Jones for language advice; and the German Federal Environmental Foundation (DBU) for funding of T.C.

Received: July 24, 2015
Revised: August 21, 2015
Accepted: August 26, 2015
Published: October 22, 2015

REFERENCES

1. Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA *78*, 3721–3725.
2. Panhuis, T.M., Butlin, R., Zuk, M., and Tregenza, T. (2001). Sexual selection and speciation. Trends Ecol. Evol. *16*, 364–371.
3. Coyne, J.A., and Orr, H.A. (2004). Speciation (Sinauer Associates).
4. Andersson, M. (1994). Sexual Selection, Monographs in Behavior and Ecology (Princeton University Press).
5. Conrad, T., Paxton, R.J., Barth, F.G., Francke, W., and Ayasse, M. (2010). Female choice in the red mason bee, *Osmia rufa* (L.) (Megachilidae). J. Exp. Biol. *213*, 4065–4073.
6. Cocroft, R.B., and Rodriguez, R.L. (2005). The behavioral ecology of insect vibrational communication. Bioscience *55*, 323–334.
7. Peters, D.S. (1978). Systematik und Zoogeographie der west-paläarktischen Arten von *Osmia* sstr, *Monosmia* und *Orientosmia*. Senckenb. Biol. *58*, 287–346.
8. Ayasse, M., Paxton, R.J., and Tengö, J. (2001). Mating behavior and chemical communication in the order Hymenoptera. Annu. Rev. Entomol. *46*, 31–78.
9. Eickwort, G.C., and Ginsberg, H.S. (1980). Foraging and mating behavior in Apoidea. Annu. Rev. Entomol. *25*, 421–446.
10. Virant-Doberlet, M., and Cokl, A. (2004). Vibrational communication in insects. Neotrop. Entomol. *33*, 121–134.
11. Klappert, K., and Reinhold, K. (2003). Acoustic preference functions and sexual selection on the male calling song in the grasshopper *Chorthippus biguttulus*. Anim. Behav. *65*, 225–233.
12. Hill, P.S.M. (2008). Vibrational Communication in Animals, First Edition (Harvard University Press).
13. Seehausen, O. (2000). Explosive speciation rates and unusual species richness in haplochromine cichlid fishes: effects of sexual selection. In Ancient Lakes: Biodiversity, Ecology and Evolution, A. Rossiter, and H. Kawanabe, eds. (Academic Press), pp. 237–274.
14. Löfstedt, C., Herrebout, W.M., and Menken, S.B.J. (1991). Sex pheromones and their potential role in the evolution of reproductive isolation in ermine moths (Yponomeutidae). Chemoecology *2*, 20–28.
15. Phelan, P.L. (1992). Evolution of sex pheromones and the role of asymmetric tracking. In Insect Chemical Ecology: An Evolutionary Approach, B.D. Roitberg, and M.B. Isman, eds. (Chapmann & Hall), pp. 265–314.
16. Seidelmann, K. (1995). Untersuchungen zur Reproduktionsbiologie der Roten Mauerbiene, *Osmia rufa* (L., 1758). PhD thesis (Halle).
17. Seidelmann, K. (1999). The race for females: the mating system of the Red Mason Bee, *Osmia rufa* (L.) (Hymenoptera: Megachilidae). J. Insect Behav. *12*, 13–25.

